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DAHLIA AS A STAIN FOR BACTERIA IN SECTIONS CUT BY THE COLLODION METHOD.

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Many elaborate methods of staining bacteria in tissues have been devised, but with nearly all of them difficulties have been encountered. Probably the greatest trouble has been in the staining of the imbedding medium or the albumen fixative which usually obscure both the tissue elements and the bacteria. Unless, therefore, the sections are cut in paraffin and not fastened to the slide by these common fixatives the bacteria are not satisfactorily brought out. Here again arises another obstacle. With loose or fragile tissues there is great danger of tearing the sections or of losing parts of them during the process of staining and dehydrating, thus destroying the value of the preparation.

Although paraffin is commonly used in pathological histology, collodion is more often employed in imbedding normal tissues. The rule in normal histology is to fasten the sections to the slide. In pathological histology they are not, for the reasons mentioned, ordinarily fastened, but in many cases it seems better to do so. The need of having an absolutely perfect section from a pathological tissue, especially for diagnosis, is even greater than is the case when sections of normal tissues are being made. The loss of a very small bit from the section may cause an entirely erroneous interpretation. By the use of collodion as the imbedding medium this danger is practically entirely eliminated, while the method is much simpler and easier than that in which paraffin is used and the sections are fastened to the slide by the use of collodion or an albumen fixative.

It is a well known fact that collodion takes most of the aniline dyes and will not give up the stain without being treated with a decolorising agent sufficiently strong to decolorise the tissue at the same time. In the case of paraffin sections which have been fastened to the slide with collodion or albumen fixative, or both, besides the disadvantage of using a process which takes a longer time, we meet the same difficulty that we did in the collodion method, in that the fixative takes the stain and obscures the preparation quite as much as does the imbedding collodion.

Both the collodion and the paraffin methods have their advantages for special kinds of work. Ordinarily in pathological histology I much prefer, for the reasons mentioned, collodion to paraffin as an imbedding medium. The method I have used is that described by Prof. S. H. Gage* in a paper read before this society in 1895. In it he summarised the whole process of sectioning by the oil-collodion method and suggested two very important improvements in the way of simplifying and cheapening the process. This method includes the improvements suggested by Dr. P. A. Fish† in 1893. Dr. Fish fastened the sections to the slide by putting a few drops of ether and alcohol on the section after it was in position. Prof. Gage used a mixture of three parts of xylene and one part of castor oil as a clarifier. In passing a section from water to strong alcohol, or vice versa, he avoids the diffusion currents by plunging the slide directly into the desired liquid instead of carrying it through successively higher or lower percentages of alcohol, as the case might be. This method, as perfected by Dr. Fish and Prof. Gage, is very simple and apparently the best one yet devised.

After finding the best method of cutting the sections the problem then seems to resolve itself into the selecting of a suitable dye that will stain the bacteria properly and yet one that will wash out of the imbedding material without the use of a decolorising agent so strong that it will remove the stain from the tissue and the bacteria.

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* S. H. Gage, Improvements in Oil-Sectioning with Collodion. Proceedings American Microscopical Society, Vol. XVII., 1895, pp. 361–370.

† P. A. Fish, A new Clearer for Collodionised Objects. Proceedings American Microscopical Society, Vol. XV., 1893, pp. 68-89

During the past year we have had a large amount of pathological material to section and for the most part for diagnosis. At first I cut most of this in paraffin, as Dr. Moore preferred it to collodion on account of the staining of the collodion. In the winter term I had some sections that I wanted to stain with gentian violet, but finding that we were out of it, I substituted dahlia in its place. These sections had been cut by the paraffin method and it was found that the stain not only showed the bacteria well but also brought out beautifully the histological structure of the tissue. Later I had occasion to cut some sections from some material which had been imbedded in collodion and to stain them for bacteria. After using other stains, such as carbol fuchsin and methyl violet, with unsatisfactory results, I tried an aqueous solution of the dahlia and found that it worked perfectly. In the process of washing and dehydrating this was entirely removed from the collodion, leaving both the tissues and the bacteria well stained and sharply differentiated.

Other formulæ, using dahlia as the dye, were tried, such as a solution containing less of the elements of a mordant nature, using 2 per cent. carbolic acid instead of 5 per cent., and also Koch-Ehrlich's aniline water solution. The carbolic acid solution did fairly well, but the aniline water solution stained the collodion too deeply and permanently. Neither brought out the cellular elements with anything like the clearness that the simple aqueous solution did.

The formula for the stain used is as follows :

Saturated alcoholic solution of dahlia	20 c.c.
Distilled water	100 c c.

The length of time necessary to stain properly varies, according to the condition of the tissue, from fifteen minutes to half an hour, that is, they must be distinctly overstained.

Then wash thoroughly with 95 per cent. alcohol until the collodion around the section appears colorless, and clear with a clearing fluid, preferably clove oil. The tissue will be well defined and the bacteria will stand out deeply stained against the more lightly stained cells of the tissue.

Of course, this method will not do with certain bacteria that require special stains or treatment, but it does work most admirably with the majority of microörganisms found in diseased animal tissues.